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Humoral Immune Response to Functional Regions of Human Cytomegalovirus Glycoprotein B

David Navarro,[1] Evelyne Lennette,[2] Sharof Tugizov,[1] and Lenore Pereira[1]*

[1]*Division of Oral Biology, School of Dentistry, University of California San Francisco, San Francisco, California*
[2]*Virolab, Berkeley, California*

Sera from patients with primary human cytomegalovirus (HCMV) infections, both acute and convalescent phase, and from HCMV-seropositive healthy subjects were analyzed to determine whether the sera would recognize antigenic domains on HCMV glycoprotein B (gB) that function in virion infectivity and spread of virus from cell to cell. The intact gB molecule, amino-terminal derivatives of different lengths, and internal deletion derivatives were expressed in eukaryotic cells and reacted by immunofluorescence with the sera. All convalescent-phase sera and most sera from healthy seropositive individuals reacted with full-length gB and with an amino-terminal derivative containing 687 amino acids (aa), gB-(1–687); approximately half of the sera recognized an amino-terminal derivative of 447 aa, gB-(1–447), and one-third reacted with the shortest deletion derivative of 258 aa, gB-(1–258). Of the acute-phase sera, 77% recognized intact gB and gB-(1–687), 32% recognized gB-(1–447), and 14% recognized gB-(1–258). Deletion of aa 548 to 618 dramatically reduced the percentage of reactive sera, whereas deletion of aa 411 to 447 had a minor impact on reactivity of sera. To investigate the epitope specificity of human antibodies to gB, we carried out competition experiments between human sera with neutralizing activity and selected monoclonal antibodies (mAbs) to conformational epitopes on gB. We found that antibodies in human sera preclude syncytium formation in UB15-11 glioblastoma cells constitutively expressing gB and compete with certain murine mAbs that block virus entry into cells and transmission of infection from cell to cell. Our results show that HCMV-immune human sera contain antibodies to functional regions on gB, and the abundance of these antibodies in convalescent-phase sera suggests that they may play a central role in limiting dissemination of virus in the host. ***J. Med. Virol. 52:451–459, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: neutralizing antibodies; syncytium formation; epitope specificity; subunit vaccine

INTRODUCTION

Human cytomegalovirus (HCMV) is a prevalent infectious agent that usually causes asymptomatic infections in healthy adults. However, HCMV is the most frequent cause of life-threatening opportunistic infection in immunosuppressed patients such as organ transplant recipients and patients infected with the human immunodeficiency virus (HIV) [Drew, 1988; Ho, 1991]. In the United States, HCMV is also the most common cause of congenital virus infection, which is associated with a high morbidity and may result in severe disease or long-term sequelae [Alford and Britt, 1993]. HCMV infections can be readily acquired in day care centers where several strains of virus circulate simultaneously [Lasry et al., 1996; Pass et al., 1982]. Development of a protective vaccine against HCMV is a long-term goal to reduce the prevalence of infection and ameliorate symptoms of disease in seronegative individuals who become infected [Adler, 1996; Plotkin, 1994; Plotkin et al., 1990].

Assays of human sera reactivity to HCMV-infected cells indicate that envelope glycoprotein B (gB) is highly immunogenic in humans [Cremer et al., 1985; Gonczol et al., 1986; Kniess et al., 1991; Liu et al., 1988; Pereira et al., 1982, 1983; Rasmussen et al., 1991] and

David Navarro is presently at Department of Microbiology, School of Medicine, University of Valencia, Spain.

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*Correspondence to: Lenore Pereira, Department of Stomatology, School of Dentistry, University of California San Francisco, 513 Parnassus Ave., San Francisco, CA 94143-0512. E-mail: pereira@itsa.ucsf.edu

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elicits antibodies with neutralizing activity [Britt et al., 1990; Britt and Vugler, 1990; Detrick et al., 1996; Rasmussen et al., 1991]. Antibodies to gB have been estimated to represent 40–70% of the total neutralizing activity in sera from HCMV-infected patients [Britt et al., 1990]. Antibody level to HCMV gB correlates with neutralizing activity and develops concurrently in transplant patients with primary infection [Marshall et al., 1992, 1994]. In addition, gB-specific lymphocyte proliferative and CD8+ and CD4+ cytolytic responses, which are important in limiting HCMV infection, are generated in infected individuals [Gonczol et al., 1990; Hopkins et al., 1996; Liu et al., 1991].

Several groups have studied the antigenic and functional structure of gB by using panels of murine and human monoclonal antibodies (mAbs) in combination with epitope-mapping strategies and functional assays [Banks et al., 1989; Basgoz et al., 1992; Britt et al., 1988; Lussenhop et al., 1988; Meyer et al., 1990, 1992; Navarro et al., 1993; Ohlin et al., 1993; Qadri et al., 1992; Utz et al., 1989; Wagner et al., 1992; Wiertz et al., 1996]. These studies indicate that certain regions of the molecule are immunodominant in humans [Kniess et al., 1991; Silvestri et al., 1991; Utz et al., 1989; Wagner et al., 1992]. Following immunization with a live HCMV vaccine or a subunit gB vaccine, both recipients have IgG and secretory IgA to HCMV gB with comparable levels of virus neutralizing activity [Wang et al., 1996]. Examination of the fine specificity of epitope recognition by antibodies to gB in sera from infected individuals will be useful in determining whether the response to a subunit gB vaccine mimics the response elicited by natural infection. In the present study, we identified the antigenic domains on gB that are recognized by HCMV-specific antibodies in human sera and examined the epitope specificity of these antibodies. Functional analysis of the neutralizing activity showed that HCMV-immune human sera preclude the formation of syncytia by UB15-11 cells expressing gB. Competition experiments with murine monoclonal antibodies (mAbs) with neutralizing activity to gB showed that sera from HCMV-infected individuals contain antibodies that recognize conformation-dependent neutralizing epitopes mapping in several antigenic domains on the molecule. These antigenic sites constitute a functional domain of gB that promotes virus penetration, cell-to-cell transmission of infection, and syncytium formation in HCMV-infected cells.

MATERIALS AND METHODS

Human Sera

Sera used in this study were the following: 23 paired sera (acute and convalescent phase) from individuals with active symptomatic HCMV infections (primary or recent reactivations), 73 sera from HCMV-infected individuals with no serological or clinical evidence of primary infection, and 12 sera from HCMV-seronegative individuals. Serological criteria of active HCMV infection were the following: detection of specific IgM and a greater than fourfold rise in the titer of specific IgG between acute- and convalescent-phase sera. Six of the paired sera without IgG antibodies to HCMV in the acute-phase serum seroconverted to HCMV and were judged to be primary infections.

Monoclonal Antibodies

Properties of the panel of mAbs to HCMV gB and the location of epitopes on gB recognized by mAbs used in this study were previously published [Basgoz et al., 1992; Meyer et al., 1992; Pereira et al., 1982, 1984; Qadri et al., 1992]. Complement-independent neutralizing mAbs to HCMV gB block virion entry into cells, cell-to-cell transmission of infection, and syncytium formation in UB15-11 cells expressing gB [Navarro et al., 1993; Tugizov et al., 1994]. The pool of mAbs to gB used in this study contained the following antibodies: CH408-1 (domain $DC1_v$, CH177-3 and CH253-1 (domain D1), CH130-9 (domain D2a), CH442-1 (domain D2b), CH409-2 (domain D3), and CH28-2 (domain DC3). A non-neutralizing mAb to gB, CH395-1 (domain D1), was used as a representative mAb that failed to compete with approximately 50% of HCMV-immune sera for binding to gB [Pereira et al., 1993].

Deletion Mutations in HCMV gB

Construction of plasmid DNAs coding full-length HCMV gB, the carboxy-terminal truncated derivatives gB-(1–258), gB-(1–447), and gB-(1–687), and the internal deletion mutants gB-(Δ411–447) and gB-(Δ548–618) was reported elsewhere [Basgoz et al., 1992; Qadri et al., 1992].

Immunofluorescence Assays

Specific antibodies to HCMV (IgM and IgG) were titrated by a conventional indirect immunofluorescence assay using HCMV-infected human foreskin fibroblasts (HFF). The humoral immune response to the different antigenic domains on gB was determined by indirect immunofluorescence reactions of human sera (1:10 dilution) with transfected COS-1 cells that transiently expressed full-length gB or deletion derivatives of the molecule. COS-1 cells were transfected with DNA of the gB-containing plasmids by the calcium phosphate coprecipitation procedure. Competition reactions, which assessed the blocking of mAb reactivity by human sera, were performed as follows: COS-1 cells transiently expressing full-length gB were reacted with human sera at 1:10 dilution in phosphate-buffered saline (PBS) at 37°C for 30 min, washed three times with PBS, and then incubated with the mAbs (1:100 in PBS, 30 min). Binding of the test mAbs was determined by incubation with goat anti-mouse IgG conjugated with FITC (1:1,000 in PBS, 30 min). After washing, slides were observed under an Olympic epifluorescence microscope. Competition was defined as complete abrogation of mAb binding to specific epitopes on gB by human sera.

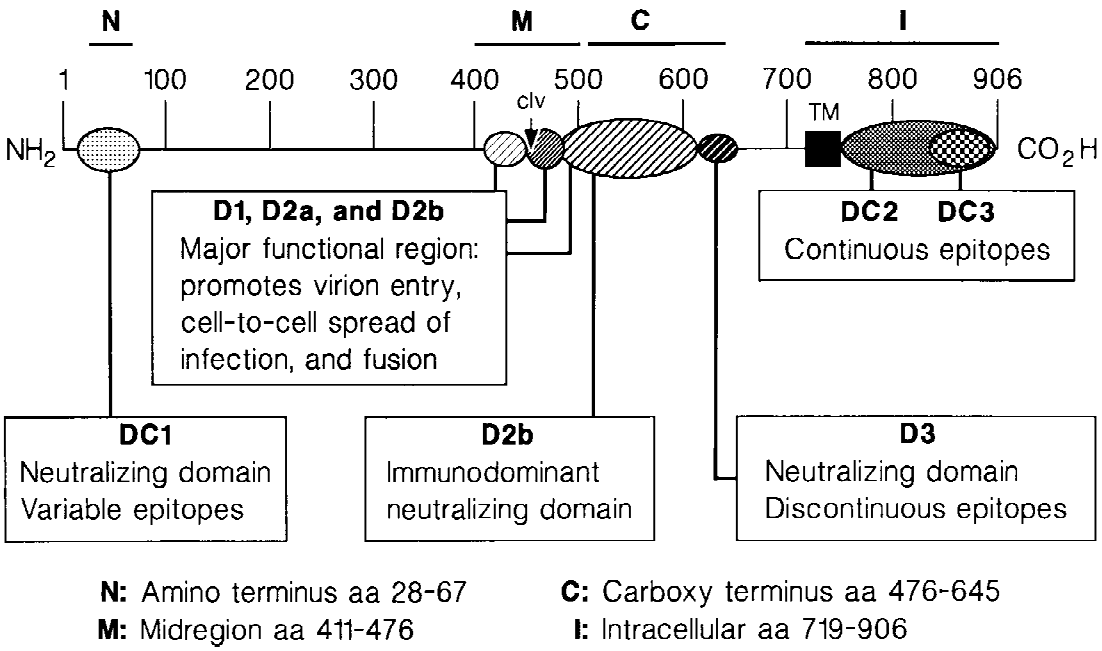

Fig. 1. Topographic map of the antigenic and functional domains on HCMV (AD169) gB (adapted from Pereira [1994]). Antigenic domains are shown as ellipses. Boxes show our designations for the antigenic domains and list their functions. Shaded box shows the transmembrane domain.

Neutralization Assays

Neutralization titers of human sera were determined by a rapid neutralization assay using HCMV strain AD169 as previously described [Andreoni et al., 1989; Navarro et al., 1993]. The neutralization titer was defined as the highest serum dilution that reduced infectivity of the input virus by 50%.

Assay for Blocking of Syncytium Formation

The ability of human sera to inhibit syncytium formation in UB15-11 glioblastoma cells that constitutively express HCMV gB was determined by a blocking assay previously described for murine mAbs [Tugizov et al., 1994]. Briefly, UB15-11 cells were grown in chamber slides, and at 6 to 10 hr postseeding the culture medium was removed and replaced with medium containing 2% fetal calf serum. Human sera from HCMV-infected individuals with neutralizing activity were added (1:100 dilution) to the cultures of gB-expressing cells. Sera from HCMV-seronegative individuals were used as a negative control and the pool of gB-specific mAbs was used as a positive control for inhibition of syncytium formation. Cells were incubated at 37°C for 24 hr, fixed with 70% methanol, stained with Giemsa for 10 min, washed, dried, and mounted with Canadian balsam. Syncytia were counted microscopically as enlarged cells containing five nuclei or more.

RESULTS

Antigenic and Functional Domains on HCMV gB

To discuss our results, we need first to refer to a topographic map of HCMV gB, which is our current model of the antigenic and functional domains on the molecule (Fig. 1, adapted from Pereira [1994]). The map shows epitopes of murine monoclonal antibodies identified by our laboratory and others [Basgoz et al., 1992; Britt et al., 1988; Kniess et al., 1991; Lussenhop et al., 1988; Masuho et al., 1987; Meyer et al., 1990, 1992; Ohlin et al., 1993; Pereira et al., 1991; Qadri et al., 1992; Schoppel et al., 1996; Utz et al., 1989; Wagner et al., 1992]. Relevant to the present study are the following: 1) domain DC1, composed of continuous neutralizing epitopes that vary among strains, maps at the extreme amino-terminus of gB; 2) domains D1, D2a and D2b, containing conformational neutralizing epitopes, map in the midregion of gB; and 3) antigenic domains D2b and D3, which contain conformational epitopes and also a linear sequence AD-1, which is highly immunogenic in humans, map in the carboxy-terminal region of gB. Domains D1, D2a and D2b are part of the functional region of gB that promotes virion entry into cells, spread of virus from cell to cell, and syncytium formation in HCMV-infected U373 cells [Navarro et al., 1993; Tugizov et al., 1994].

Reactivity of Human Sera With gB and Deletion Derivatives

In the first set of experiments, we determined whether sera from patients with active HCMV infections and from seropositive individuals would recognize specific antigenic domains on gB. Human sera were reacted by immunofluorescence with COS-1 cells that transiently expressed intact gB and deletion derivatives lacking different lengths of the carboxy terminus or internal sequences in the midregion of gB. Each derivative had

TABLE I. Human Sera Reactivity With HCMV gB and Deletion Derivatives of gB

Antigen[a]	Active symptomatic infection (n = 23) Acute phase (% reactive)	Active symptomatic infection (n = 23) Convalescent phase (% reactive)	Seropositive (n = 73) (% reactive)
Full-length gB	77	100	96
gB-(1–687)	77	100	97
gB-(1–447)	32	60	53
gB-(1–258)	14	30	32
gB-(Δ411–447)	70	100	ND
gB-(Δ548–618)	24	30	ND

[a]Construction and antigenic properties of deletion derivatives were described [Qadri et al., 1992; Tugizov et al., 1995].

TABLE II. Functional Properties of Complement-Independent Neutralizing Antibodies to HCMV gB

Monoclonal antibody	Antigenic domain	CMV gB functions blocked: Cell-to-cell spread of virus[a]	CMV gB functions blocked: Fusion of infected U373 cells[b]
CH177-3	D1	+++	+++
CH253-1	D1	++	++
CH358-5	D1	−	−
CH382-2	D1	++	++
CH388-4	D1	++	++
CH130-9	D2a	++	++
CH143-13	D2a	++	++
CH432-1	D2b	+	++
CH442-1	D2b	+++	++
CH446-2	D2b	++	++
CH436-1	D2b	++	++

[a]Cell-to-cell spread blocked with 100 μg/ml antibody. Nuclei in 20–30 plaques were counted. +++, >75% inhibition when compared to wild-type plaques; ++, 50–75% inhibition; +, 25–50% inhibition; −, no reduction in plaque size [Navarro et al., 1993].
[b]Fusion of CMV-infected cells (1 PFU/cell, 4 days postinfection, blocked with 50 μg/ml): +++, 75%; ++, 50%; −, <25% [Navarro et al., 1993].

been previously characterized for antigenic properties by reaction with a panel of mAbs with known epitope specificities [Basgoz et al., 1992; Qadri et al., 1992; Tugizov et al., 1995]. Specificity of the assay was assessed by reacting sera from 12 HCMV-seronegative individuals with COS-1 cells expressing gB. None of these sera reacted with gB-expressing cells.

Table I shows the reactivity of sera with full-length gB, truncated derivatives, and the internal deletion derivatives of gB. All convalescent-phase sera from patients with active infections and most sera (96%) from seropositive individuals reacted with cells expressing the full-length gB molecule. A similar reactivity pattern was obtained with the amino-terminal derivative gB-(1–687). Likewise, comparable percentages of acute-phase sera reacted with gB and gB-(1–687), although the overall number of reactive sera binding to gB was lower than that of convalescent-phase sera and sera from seropositive individuals. This result indicated that immunodominant epitopes on gB recognized by human antibodies elicited during HCMV infection are contained in the deletion mutant gB-(1–687). The percentages of sera reacting with shorter gB truncation derivatives were substantially lower, yet more than half the convalescent sera (60%) and sera from non-acutely infected individuals (53%) reacted with the truncated derivative containing the amino-terminal 447 aa. Approximately one-third (32%) of the seropositive individual sera reacted with the shortest gB derivative, gB-(1–258). Sera that reacted with the shorter derivatives also recognized the longer ones.

We previously reported that mAbs binding to conformation-dependent epitopes in domain D1 failed to bind gB-(Δ411–447) [Tugizov et al., 1995] and also found that deletion of aa 548 to 618 abrogated recognition by mAbs to conformation-dependent epitopes in domain D2b (S. Tugizov and L. Pereira, unpublished data). In the next series of experiments, we reacted the human sera with internal deletion derivatives gB-(Δ411–447) and gB-(Δ548–618) to determine whether the conformation of these regions was required for recognition (Table I). Deletion of aa 411 to 447 had no effect on reactivity of sera, but deletion of the sequence containing the carboxy-terminal aa 548 to 618 dramatically abrogated the reactivity of most sera (75%), including that from patients with primary infections. This finding indicated that proper conformation of domain D2b in the carboxy-terminal half of gB is required for recognition by human sera.

Neutralizing Functions of Antibodies to gB in Human Sera and Competition With mAbs to Conformational Epitopes

To determine whether sera from HCMV-infected individuals contain antibodies with functions and epitope specificities similar to those of murine mAbs, we examined the neutralizing functions of the sera and tested their ability to compete for binding to gB with complement-independent neutralizing murine mAbs. These mAbs recognized conformational epitopes in antigenic regions D1, D2a and D2b, which constitute a region important for gB function (Table II). They neutralized virus infectivity by abrogating entry of HCMV virions into cells, syncytium formation in UB15-11 cells, and cell-cell spread of virus in HFF and U373 glioblastoma cells [Navarro et al., 1993; Tugizov et al., 1994]. Thirteen human sera were chosen from those reacting with full-length gB and gB-(1–447) to be tested in these experiments (five acute-phase IgM-positive sera and eight convalescent-phase sera). These human sera had neutralizing titers ranging from 1:100 to 1:800 in neutralization tests with free virus (Table III). In reactions with UB15-11 cells expressing full-length HCMV gB that induced syncytium formation (Fig. 2A), antibodies in the sera with neutralizing activity reduced the formation of syncytia (Fig. 2B). Results obtained with the HCMV immune sera were comparable to syncytium reduction observed with neutralizing murine mAbs (Fig. 2C). These findings are important because they show that human serum antibodies have functional activi-

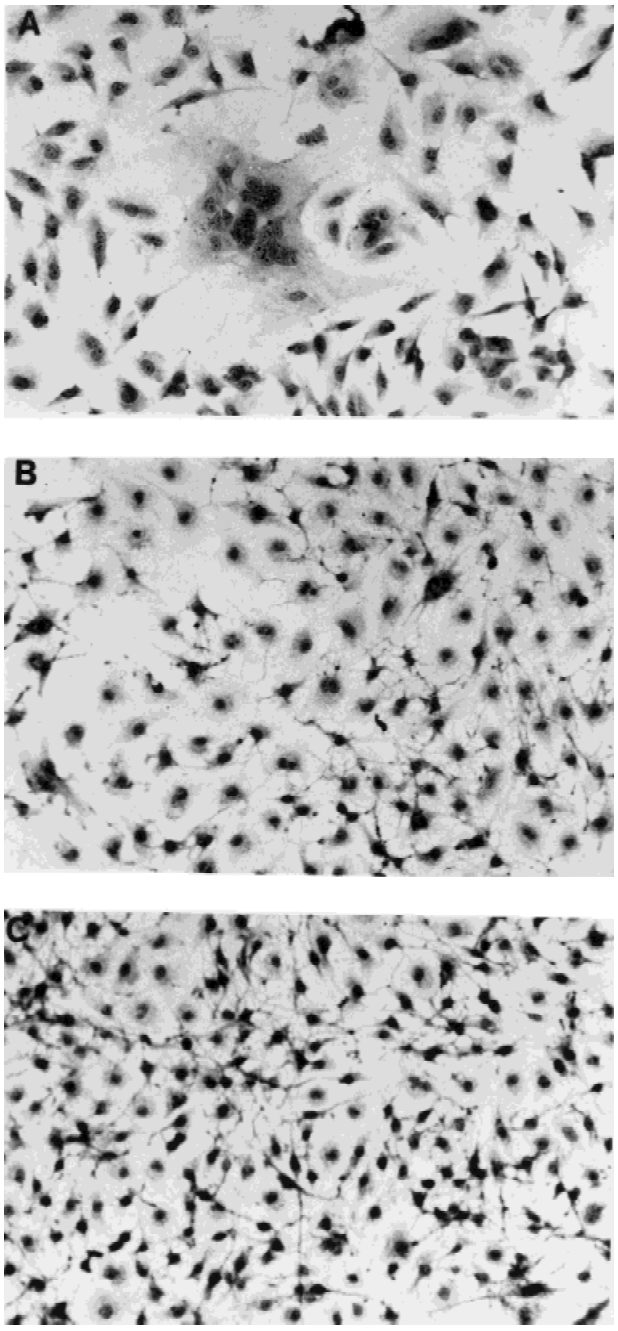

Fig. 2. Fusion of UB15-11 glioblastoma cells constitutively expressing HCMV (AD169) gB that form syncytia **(A)** and blocking of gB-induced syncytium formation by HCMV immune serum no. 1 **(B)** and by pool of murine neutralizing mAbs reactive with HCMV gB **(C).** Magnification, 250×

ties similar to neutralizing murine mAbs to HCMV gB. Results of these experiments are summarized in Table III.

Next, we carried out competition experiments with human sera and murine mAbs to HCMV gB. In these experiments, competition was defined as complete abrogation of the mAb binding by human sera as shown in immunofluorescence reactions with gB-expressing cells. Shown in Figure 3 are reactions of non-neutralizing mAb CH395-1 to gB (panel A) and in the presence of a non-competitive HCMV immune serum (panel B), and reactions of neutralizing mAb CH436-1 (panel C) and in the presence of a competitive human serum that completely prevents binding of the murine mAb to gB (panel D). Data with a panel of mAbs to gB in competition reactions with immune sera to HCMV are summarized in Table IV. Of the five acute-phase sera tested, only two contained antibodies that competed with murine mAbs; one of them (serum 6) showed competition with three mAbs recognizing epitopes in D2a and D2b, and the other (serum 7) competed with six mAbs recognizing epitopes in D1, D2a and D2b. The remaining acute-phase sera were unable to compete with any of the mAbs included in the competition experiments. All convalescent-phase sera tested competed with at least five mAbs binding epitopes in D1, D2a and D2b. Competition of human sera with mAbs CH388-4, CH130-9 and CH143-13 was the most frequent pattern (Table IV). Inasmuch as patient sera failed to compete with mAbs CH177-3 and CH253-1 to epitopes in domain D1, recognition of these sites may be specific to antibodies elicited in mice. Sera from HCMV-seronegative individuals failed to compete with any of the mAbs tested. These results indicated that antibodies with epitope specificities and functional activities similar to neutralizing murine mAbs are generated in HCMV-infected patients.

DISCUSSION

In this study, we examined the human antibody response to different antigenic domains on HCMV gB in patients with primary HCMV infections and in seropositive individuals without disease. Antibodies to full-length gB were present in all convalescent-phase sera from patients with primary infections and in virtually all sera from seropositive individuals. These findings are in agreement with earlier reports [Britt et al., 1990; Cremer et al., 1985; Detrick et al., 1996; Gonczol et al., 1990; Pereira et al., 1982; Rasmussen et al., 1991] and further support the idea that gB is a potent immunogen in humans. Antibodies that recognize gB were present in almost 80% of acute-phase sera from patients with primary HCMV infections, indicating that in most cases, antibodies to gB appear soon after infection and thereby serve as a marker of seroconversion. We showed that reactivity of human sera with the truncated derivative gB-(1–687) paralleled their reactivity with full-length gB. This finding confirms that the immunodominant epitopes on gB are located in the ectodomain of the molecule, which is consistent with the topography of gB in the membrane of infected cells [Basgoz et al., 1992] and indicates that deletion of the transmembrane and intracellular regions of gB does not alter the binding of seropositive sera to gB as previously reported for murine mAbs [Qadri et al., 1992]. The percentage of sera reacting with gB-(1–447), which contains antigenic domains DC1 and D1 but lacks the carboxy-terminal half of the molecule, was lower in sera from primary infections and in sera from seropos-

TABLE III. Neutralizing Titer and Syncytium-blocking Activity of Patient Sera that Compete With Murine mAbs for HCMV gB Binding

Serum	IgM	Neut. titer[a]	Syn./cm^{2}[b]	Syn. reduction[c]
1	+	1:800	18/30	88.0
4	+	1:800	21/29	87.5
5	+	1:400	20/40	82.8
6	+	1:800	22/31	86.6
7	+	1:200	17/26	89.2
16	−	1:200	31/44	81.2
45	−	1:200	40/37	80.7
47	−	1:100	90/67	60.5
56	−	1:800	24/33	85.6
58	−	1:800	49/34	79.3
59	−	1:200	91/74	58.8
82	−	1:100	41/64	73.6
91	−	1:100	56/79	66.3
gB mAb pool		reported [Navarro et al., 1993]	40/44	79.0
Seronegative 1		0	194/206	0
Seronegative 2		0	189/191	0
No serum		0	221/204	0

[a]Neutralization titer.
[b]Number of syncytia/cm^{2} in U373 cells expressing HCMV gB following treatment with sera and gB mAbs (samples run in duplicate).
[c]Mean syncytium reduction.

itive individuals with past infections, indicating that relevant epitopes recognized by human antibodies had been deleted or the molecule had been altered in conformation, or both. The percentage of sera reacting with gB-(1–258) was substantially lower (30% of IgM-negative sera). Two antigenic sites containing linear epitopes that are recognized by human and murine neutralizing antibodies map within this region of gB (DC1) [Basgoz et al., 1992; Masuho et al., 1987]. One site contains strain-variable epitopes and has been located between amino acids 50 and 54 [Meyer et al., 1990, 1992]. The other antigenic site maps between amino acids 68 and 84; it is highly conserved among HCMV strains and is recognized by human mAbs with complement-independent neutralizing activity. However, it was recently reported that approximately half of the human sera tested reacted with a fusion protein constructed to express these amino acid sequences [Ayata et al., 1994]. Results of the present study support the idea that the strain-common epitopes located in the extreme amino-terminal sequence of gB elicit antibodies in some, but not all, HCMV-infected individuals.

Experiments in which human sera were reacted with internal deletion derivatives of gB yielded interesting data. We found that deletion of aa 411 to 447 did not preclude reactions of human sera with the derivative gB-(Δ411–447). Interestingly, this region of gB contains conformational epitopes recognized by potent complement-independent neutralizing mAbs that block fusion in UB15-11 cells expressing gB [Tugizov et al., 1994]. That this region of gB promotes virion entry into U373, HFF, and polarized retinal pigment epithelial cells, and cell-cell spread of virus in nonpolarized cells [Navarro et al., 1993; Tugizov et al., 1996], led us to suggest that it formed part of the major functional domain of the molecule. We subsequently found [Tugizov et al., 1995] that a cell line constitutively expressing gB-(Δ411–447) behaved like cells expressing the wild-type molecule in terms of spontaneous syncytium formation, which indicates that the conformation of this region is not critical for the functional activity of gB. Results of the present study indicate that although antibodies to epitopes in domain D1 were elicited during HCMV infection, they represent a fraction of the total gB-specific antibodies as indicated by the reactivity of 60% of convalescent sera with gB-(1–447) and 100% with gB-(1–687). Moreover, deletion of aa 548 to 618 dramatically reduced the percentage of reactive sera down to 30% (Table I). The amino acids removed in the latter derivative of gB are assembled into domain D2b, which comprises conformational epitopes also recognized by murine mAbs [Qadri et al., 1992]. Likewise the antigenic linear site AD-1, which requires a minimal sequence for binding of specific antibodies encompassing aa 552–638 [Britt et al., 1990, 1988; Kniess et al., 1991; Utz et al., 1989; Wagner et al., 1992], maps in this region of the molecule. Failure of murine mAb-17 to recognize our deletion derivative gB-(Δ548–618) showed that this mAb recognized an epitope in domain D2b (L. Pereira, unpublished data). Antigenic site AD-1 binds both human and murine complement-independent and complement-dependent neutralizing antibodies, is highly conserved among HCMV isolates, and is recognized by sera from a large number of HCMV-infected individuals [Kniess et al., 1991; Ohlin et al., 1993; Utz et al., 1989; Wagner et al., 1992]. Our results showed that the native conformation of domain D2b is required for recognition of gB by human sera, whereas altered conformation of domain D1 does not substantially impair recognition. The finding that 100% of the convalescent sera react with gB-(Δ411–447) whereas 60% react with gB-(1–447) and only 30% with gB-(Δ548–618) suggests that deletion of aa 548–

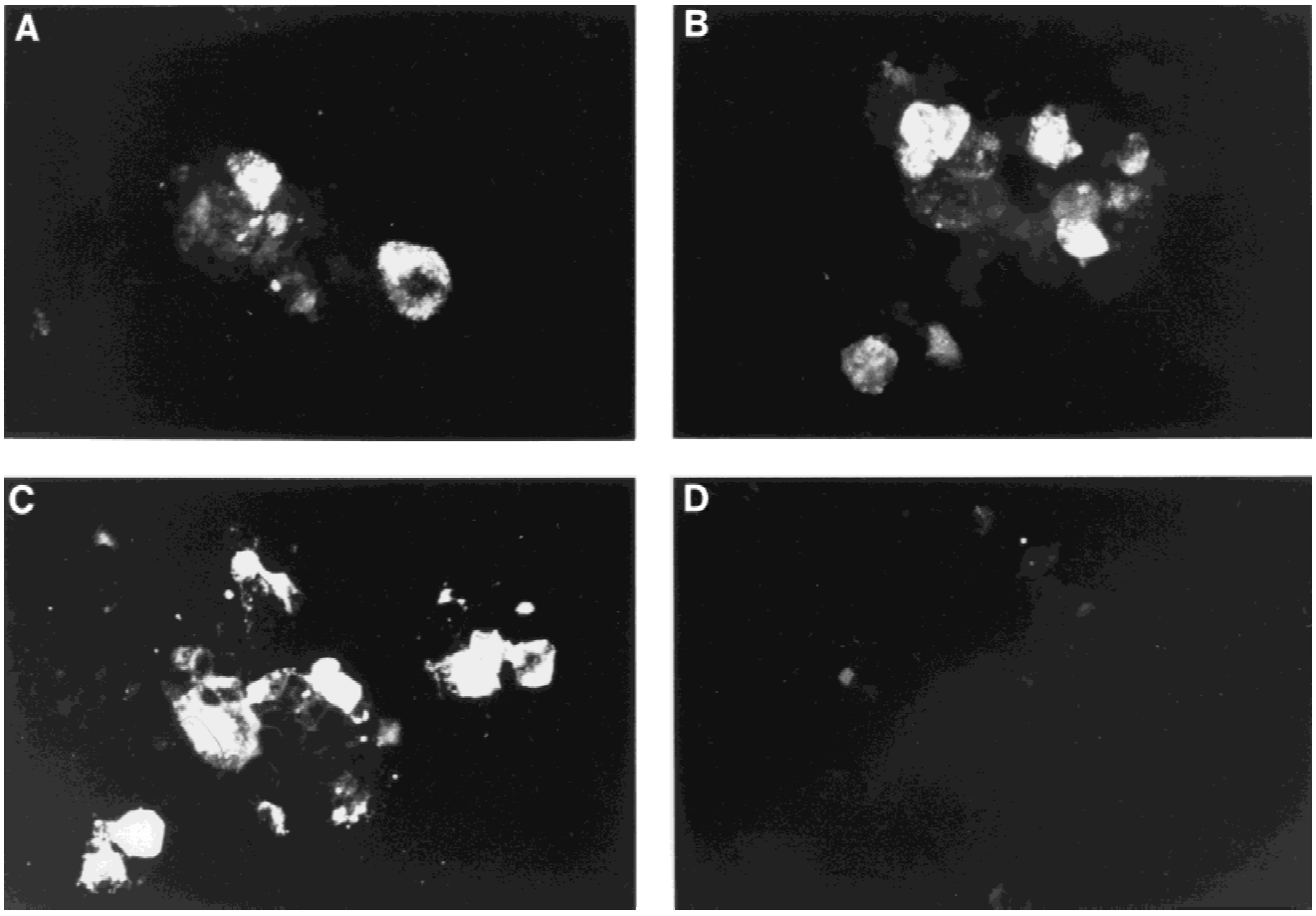

Fig. 3. Immunofluorescence photomicrographs illustrating competition between HCMV immune human sera and murine mAbs for binding to HCMV gB. Shown is the reactivity of mAbs to gB and serum samples in reactions with transfected COS-1 cells expressing full-length gB. Non-neutralizing mAb CH395-1 to domain D1 **(A),** in the presence of non-competitive human serum **(B),** neutralizing mAb CH436-1 to domain D2b **(C),** in the presence of competitive human serum **(D).** Magnification, 250×.

TABLE IV. Competition Between Patient Sera and Complement-independent Neutralizing Murine mAbs for Binding to HCMV gB

		Competition	
mAb[a]	Domain on gB[b]	No. pos./total	%
CH177-3	D1	0/13	0
CH253-1	D1	0/13	0
CH358-5	D1	8/13	61.5
CH382-2	D1	6/13	46.1
CH388-4	D1	9/13	69.2
CH130-9	D2a	9/13	69.2
CH143-13	D2a	9/13	69.2
CH432-1	D2b	5/13	38.4
CH442-1	D2b	5/13	38.4
CH446-2	D2b	5/13	38.4
CH436-1	D2b	6/13	46.1

[a]Properties reported [Navarro et al., 1993; Qadri et al., 1992; Tugizov et al., 1994].
[b]Mapping of epitopes recognized by mAbs was previously reported [Basgoz et al., 1992; Qadri et al., 1992].

618 alters the antigenic properties of distal conformation-dependent epitopes mapping within aa 411–447 of the membrane-anchored form of gB.

To address the epitope specificities of human antibodies elicited in HCMV-infected individuals, we performed competition experiments testing the ability of human sera to block binding of selected murine mAbs with neutralizing activity. Human sera used in competition experiments displayed important antiviral properties, including neutralization of free virus and inhibition of syncytium formation in UB15-11 cells that express HCMV gB. This indicated that serum antibodies perform antiviral activities similar to those of murine mAbs to gB. Convalescent-phase sera from individuals recovering from a primary infection blocked binding of one or more mAbs recognizing conformational epitopes assembled in antigenic domains D2a and D2b, which form the major functional domain of the molecule [Navarro et al., 1993; Tugizov et al., 1994, 1995]. Three of five acute-phase sera from individuals with primary infections failed to compete with any mAbs in our panel, suggesting that in some individuals the appearance of gB-specific antibodies with important antiviral activities might be delayed or less abundant. However, we cannot exclude the possibility that functional antibodies of different specificities from those tested appear early after HCMV infection. Al-

though competition from steric hindrance cannot be completely ruled out, this possibility seems unlikely, insofar as sera blocked the binding of certain mAbs but not of others that recognized closely located epitopes in the molecule. Interestingly, all human sera tested failed to compete with two potent neutralizing mAbs (CH177-3 and CH253-1), suggesting that a small subset of epitope specificities of human and murine mAbs may differ.

Our results showed that most of the antibodies generated in HCMV-infected individuals have epitope specificities similar to those of murine mAbs; further, they suggest that, like these murine mAbs, neutralizing antibodies to gB in patient sera inhibit virion entry and transmission of infection. These antiviral functions of antibodies appear to be central in preventing virus dissemination to multiple organ sites after primary HCMV infection or reactivation. We recently reported that HCMV gB-specific neutralizing mAbs that blocked virus entry and spread of virus from cell to cell in fibroblasts failed to inhibit intercellular transmission of virus in polarized retinal pigment epithelial (RPE) cells [Tugizov et al., 1996]. Although the antibodies block entry of HCMV virions occurring through the apical surface of polarized RPE cells grown on permeable filter supports, they fail to inhibit virus spread across lateral membranes [Tugizov et al., 1996]. Our observations in cultured RPE cells suggest that neutralizing antibodies to gB in human sera may not preclude virus spread in the retina or in tissues comprised of epithelial cells. Recent progress establishing the SCID-Hu mouse for propagation of HCMV in tissue implants of human thymic epithelial cells provides a potential animal model to evaluate the effect of mAbs to gB and other HCMV glycoproteins that elicit virus neutralizing activity [Brown et al., 1995; Mocarski et al., 1993]. It was recently reported that HCMV isolates from infected patients contain 22 additional open reading frames predicted to encode glycoproteins, which may explain the differences these strains exhibit in virulence and tissue tropism in the SCID-Hu mouse model [Cha et al., 1996]. Based on these findings, it remains to be determined whether mAbs with potent neutralizing activity to HCMV gB or other glycoproteins will limit virus spread in vivo.

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